

## Degradation of Immunoglobulins A1, A2, and G by Suspected Principal Periodontal Pathogens

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Attention has recently been focused on immunoglobulin A1 (IgA1) protease production as a possible virulence factor of bacteria implicated in meningitis and gonorrhoea. This report demonstrates that suspected principal etiological agents in destructive periodontal disease include bacteria capable of degrading IgA1, IgA2, and IgG. Representative strains of *Bacteroides melaninogenicus* subsp. *melaninogenicus* and *Capnocytophaga* cleaved IgA1 but not IgA2 in the hinge region to yield intact Fab and Fc fragments. All *Capnocytophaga* strains also cleaved IgG in the same way. The majority of strains of *Bacteroides asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius* caused complete degradation of both IgA1 and polyclonal IgG. However, some strains left the Fc part of IgA1 intact. Several strains were also capable of completely decomposing IgA2 and S-IgA. Significant IgA-cleaving enzyme activity was detected in whole subgingival dental plaque collected from patients with destructive periodontal disease. The results indicate that colonization of the subgingival area by *B. asaccharolyticus*, *B. melaninogenicus*, and *Capnocytophaga* spp. can induce a local paralysis of the immune defence mechanisms, thereby facilitating the penetration and spread of potentially toxic substances, lytic enzymes, and antigens released by the entire subgingival microflora.

Periodontal disease is an inflammatory reaction in the gingival tissues which takes place as a response to the penetration of products of bacteria accumulating in the subgingival areas around the teeth. It has been estimated that periodontal disease is one of the most frequently occurring infectious diseases affecting humans. The disease can taken relatively mild or severe forms but almost invariably leads to breakdown of the supporting tissues of the teeth.

Microbial communities associated with periodontal disease have only been described in any detail relatively recently. As a result of the application of improved methods for isolation and classification of oral microorganisms, certain groups of bacteria have been shown to be associated with the various forms of destructive periodontal disease (18, 19, 28, 29, 31, 33). Collectively, these studies have implicated the following relatively small group of gram-negative, anaerobic, or capnophilic rods as principal etiological agents in destructive periodontal disease: *Bacteroides asaccharolyticus*, *Bacteroides melaninogenicus*, *Capnocytophaga ochracea*, *Actinobacillus actinomycetemcomitans*, and possibly some unclassified gram-negative anaerobic rods.

Immunoglobulin A (IgA) is generally accepted as the principal immunological barrier to inva-

sion of human mucosal surfaces by bacteria, viruses, and other pathogens. Furthermore, correlations between selective IgA deficiency and certain immune complex-mediated diseases, as well as results of several experimental studies of antigenic penetration, have suggested that the IgA system stands as a barrier against the absorption and effective antigenicity of the myriad of antigens of nutritional and microbial origin to which the body is constantly being exposed (9). In view of this, it was of considerable interest that the three leading causes of bacterial meningitis, *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*, and the etiological agent in gonorrhoea, *Neisseria gonorrhoeae*, were shown to produce an enzyme (IgA protease), which specifically cleaves human IgA subclass 1 (IgA1) to yield intact Fab and Fc fragments (14, 15, 21). The fact that closely related nonpathogenic bacteria lack this property (14, 17) strongly suggests that IgA protease activity may be an important virulence factor of bacteria attacking mucosal surfaces. IgA protease activity has also been demonstrated in some strains of the two streptococcal species, *S. sanguis* and *S. mitior*, which are responsible for the initiation of bacterial accumulation on dental surfaces (12, 20).

Recently, I undertook a comprehensive

screening of bacteria and mycoplasma for IgA1 protease activity to reach a more complete understanding of the occurrence of this property in microorganisms of medical importance (M. Kilian, submitted for publication). The study included 625 strains representing the genera *Actinobacillus*, *Actinomyces*, *Bacteroides*, *Bordetella*, *Capnocytophaga*, *Clostridium*, *Corynebacterium*, *Eikenella*, *Escherichia*, *Eubacterium*, *Flavobacterium*, *Fusobacterium*, *Klebsiella*, *Legionella*, *Leptotrichia*, *Listeria*, *Moraxella*, *Mycobacterium*, *Mycoplasma*, *Pseudomonas*, *Salmonella*, *Shigella*, *Streptococcus*, *Veillonella*, *Vibrio*, and *Yersinia*. In addition to previously recognized producers of IgA1 protease, only strains of the suspected principal etiological agents in periodontal disease, *B. asaccharolyticus*, *B. melaninogenicus*, and the three *Capnocytophaga* species, were found to be capable of cleaving IgA1. This finding is the subject of the present report, which furthermore demonstrates that some of these microorganisms cleaved IgA2 and IgG.

#### MATERIALS AND METHODS

**Bacterial strains and culture conditions.** The 45 strains of *Bacteroides* and *Capnocytophaga* used in the study are listed in Table 1. All strains were cultivated on a tryptone-yeast extract agar supplemented with hemolyzed blood, menadione, heme, and cysteine (11) and were incubated in an atmosphere of 5% CO<sub>2</sub>, 85% N<sub>2</sub>, and 10% H<sub>2</sub>.

**Immunoglobulin preparations.** Purified preparations of human IgA1 (Car) and IgA2 (Fel) paraproteins and colostral S-IgA were a generous gift of J. Mestecky, University of Alabama in Birmingham. Polyclonal IgG was isolated from normal human serum (6).

**Detection of cleavage of immunoglobulins.** Individual bacterial colonies from 3- to 4-day agar plate cultures were suspended in 40  $\mu$ l of a 2- to 5-mg/ml solution of purified immunoglobulin in 0.05 M Tris(hydroxymethyl)aminomethane - hydrochloride buffer, pH 7.4, containing 0.85% (wt/vol) sodium chloride. After incubation for 18 h at 35°C, proteolysis was detected by immunoelectrophoresis and by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. For reference purposes, IgA1 paraprotein cleaved with IgA1 protease isolated from a strain of *H. influenzae* was used (14).

**Immunoelectrophoresis.** Immunoelectrophoresis of digested proteins and undigested controls was performed in 2% Special Noble agar (Difco Laboratories, Detroit, Mich.) in Veronal buffer, pH 8.6. Antisera were unabsorbed rabbit antiserum raised against human colostral S-IgA and commercial rabbit antisera against human immunoglobulins,  $\alpha$ -chain of IgA,  $\kappa$ -type light chain (DAKO-immunoglobulins, Copenhagen), and Fab and Fc fragments of human IgG (Behringwerke AG, Marburg, Germany).

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.** Samples were electrophoresed in

0.1 M phosphate buffer, pH 7.1, in the presence of 0.1% sodium dodecyl sulfate and 5 M urea with the Multiphor system (LKB Instruments, Inc., Bromma, Sweden). For cleavage of disulfide bridges, some samples were heated in a boiling water bath for 2 min in the presence of 0.2 M 2-mercaptoethanol-5 M urea-1% sodium dodecyl sulfate. Apparent molecular weights of fragments observed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis were calculated (40), and the following proteins were used as standards: phosphorylase A (100,000 molecular weight), bovine serum albumin (68,000 molecular weight), catalase (58,000 molecular weight), and myoglobin (17,200 molecular weight). The molecular weights of fragments in reduced samples were determined with reference to reduced and purified L- and H-chains of myeloma IgG.

#### RESULTS

**Cleavage of immunoglobulins.** Immunoelectrophoretic analysis of IgA1 paraprotein incubated with the 45 strains of *Bacteroides* and *Capnocytophaga* revealed that all but five strains caused degradation of IgA1 to various extents. The five exceptions included two strains of *B. asaccharolyticus*, one strain of *B. melaninogenicus* subsp. *melaninogenicus*, and two strains of *B. melaninogenicus* subsp. *intermedius* (Table 1). Among the remaining 40 strains, three distinct patterns of IgA1 cleavage were apparent. All strains of *B. melaninogenicus* subsp. *melaninogenicus* and the *Capnocytophaga* species and one strain each of *B. asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius* cleaved IgA1 to yield a pattern resembling that of IgA1 digested with *H. influenzae* IgA1 protease (Fig. 1). A second pattern was observed with IgA1 that had been incubated with four strains of *B. asaccharolyticus* (ATCC 25260, VPI 4199, B 536, and B537) and one strain of *B. melaninogenicus* subsp. *intermedius* (NCTC 9336). Immunoelectrophoresis of IgA1 incubated with these five strains yielded one precipitation line, which showed an electrophoretic mobility significantly different from that of uncleaved IgA1 (Fig. 1). The third pattern was observed with the remaining 6 strains of *B. asaccharolyticus* and with 8 of the 10 strains of *B. melaninogenicus* subsp. *intermedius* (Table 1). IgA1 incubated for 18 h with these strains showed no or very faint reactivity with antisera against S-IgA or  $\alpha$ - or  $\kappa$ -chains. Hourly examination of IgA1 incubated for 1 to 24 h with these strains revealed a gradually progressing disappearance of antigenic reactivity (Fig. 2). Five strains each of *B. asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius* (Table 1), which caused complete degradation of IgA1, were also capable of degrading IgA2 paraprotein to the extent that no antigenic reac-

TABLE 1. Degradation of immunoglobulins A1, A2, and G by strains of *B. asaccharolyticus*, *B. melaninogenicus*, and *Capnocytophaga*

Strain	Source <sup>a</sup>	Site of isolation	Cleavage of immunoglobulins <sup>b</sup>		
			IgA1	IgA2	IgG
<i>B. asaccharolyticus</i>					
ATCC 25260	ATCC	Empyema	Fc re- maining		Complete
NCTC 9337	NCTC	Infected hemorrhoids	Complete		Complete
W 83	Shah ← Werner	Clinical specimen	Complete	Complete	Complete
2848	Shah ← Sutter	Perforated appendix			
VPI 4199	Shah ← Sutter	Human feces	Fc re- maining		Complete
B 536	Shah ← Sutter	Human feces	Fc re- maining		Complete
B 537	Shah ← Sutter	Human leg lesion	Fc re- maining		Complete
102	Palenstein ← Socransky	Periodontal disease	Fab/Fc		Complete
AN 1682/8	Justesen	Vaginal secretion			
B 37an	My own isolate	Periodontal disease	Complete	Complete	Complete
D 16an	My own isolate	Periodontal disease	Complete	Complete	Complete
BN 11 a-f	Sundquist	Infected root canal			
HN 11 a-e	Sundquist	Infected root canal	Complete	Complete	Complete
TN 42 -c	Sundquist	Gingival crevice	Complete	Complete	Complete
<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i>					
ATCC 25845	ATCC	Human sputum	Fab/Fc		
ATCC 15930	ATCC	Gingival crevice	Fab/Fc		
D8	Palenstein ← Socransky	Periodontal disease			
D23	Palenstein ← Socransky	Periodontal disease	Fab/Fc		
D34	Palenstein ← Socransky	Periodontal disease	Fab/Fc		
379	Palenstein ← Socransky	Periodontal disease	Fab/Fc		
NCTC 11321	NCTC	?	Fab/Fc		
R46508/80	Justesen	Abscess	Fab/Fc		
<i>B. melaninogenicus</i> subsp. <i>intermedius</i>					
ATCC 25261	ATCC	Laryngotomy wound	Complete	Complete	Complete
NCTC 9336	NCTC	Vincent's gingivitis	Fc re- maining		Complete
NCTC 9338	NCTC	Empyema	Complete		Complete
A 450	Palenstein ← Socransky	Periodontal disease	Complete		Complete
A 452	Palenstein ← Socransky	Periodontal disease	Complete	Complete	Complete
A 453	Palenstein ← Socransky	Periodontal disease	Complete	Complete	Complete
R62521/80	Justesen	Submandibular abscess	Complete		Complete
AN 1996	Justesen	Mandibular abscess			
VJB 13-c	Sundquist	Infected root canal	Complete	Complete	Complete
AB13-af	Sundquist	Infected root canal	Complete	Complete	Complete
P-K	Sundquist	Infected root canal	Fab/Fc		
<i>Capnocytophaga</i> sp.					
ATCC 27872 ( <i>C. ochracea</i> )	ATCC	Gingival crevice	Fab/Fc		Fab/Fc
2010 ( <i>C. ochracea</i> )	Hardie ← Socransky	Gingival crevice	Fab/Fc		Fab/Fc
209	Hardie ← Slots	Gingival crevice	Fab/Fc		Fab/Fc
6100	Hardie ← CDC	?	Fab/Fc		Fab/Fc
2467b	Hardie ← Duerden	?	Fab/Fc		Fab/Fc
25 ( <i>C. ochracea</i> )	Holt	Gingival crevice	Fab/Fc		Fab/Fc
VK 17	My own isolate	Periodontal disease	Fab/Fc		Fab/Fc
VK 18	My own isolate	Periodontal disease	Fab/Fc		Fab/Fc
AM 648/102	Hardie	Dental plaque	Fab/Fc		Fab/Fc
AM 10753	Hardie	Dental plaque	Fab/Fc		Fab/Fc
4 ( <i>C. sputigena</i> )	Holt	Gingival crevice	Fab/Fc		Fab/Fc
30N-51 ( <i>C. gingivalis</i> )	Holt	Gingival crevice	Fab/Fc		Fab/Fc

<sup>a</sup> ATCC, American Type Culture Collection, Rockville, Md.; Hardie, J. Hardie, London Hospital Dental School, London; Holt, S. C. Holt, University of Massachusetts, Amhurst; Justesen, T. Justesen, University of Copenhagen; NCTC, National Collection of Type Cultures, Colindale; Palenstein, W. Palenstein Helderman, University of Utrecht, Dental Institute, Utrecht, The Netherlands; Sundquist, G. Sundquist, University of Umeå, Dental Faculty, Umeå, Sweden. Arrows indicate something.

<sup>b</sup> Fc remaining, Fc part of immunoglobulin remained intact, whereas Fab part was completely degraded; complete, complete degradation of immunoglobulin into small-molecular-weight fragments; Fab/Fc, cleavage of immunoglobulin in hinge region to yield intact Fab and Fc fragments.

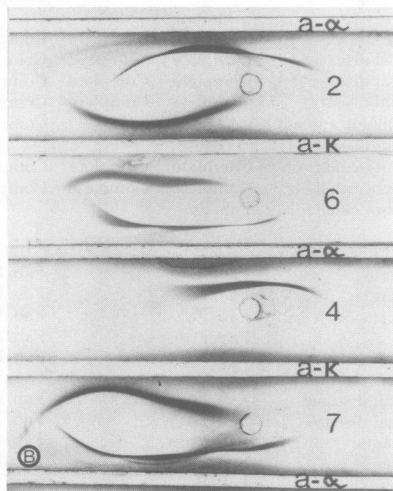
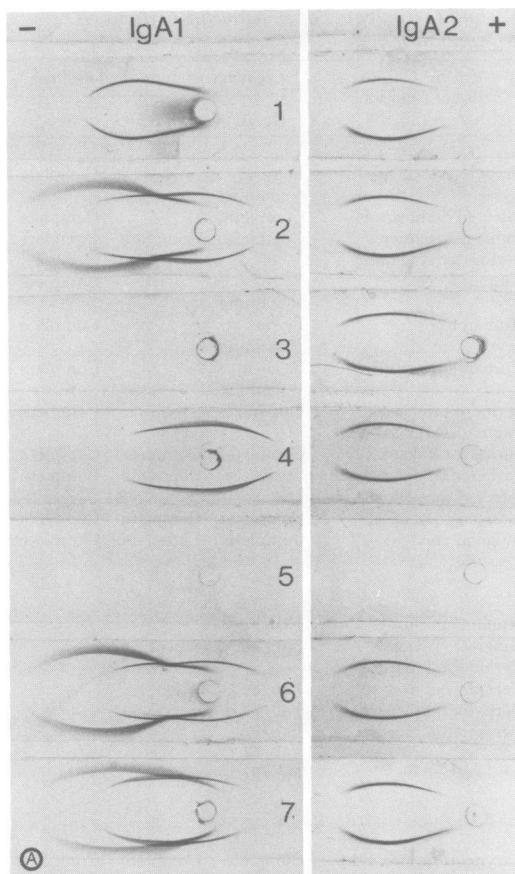


FIG. 1. Immunoelectrophoretic pattern of IgA1 and IgA2 paraproteins after incubation with buffer (1), and with cells of *B. melaninogenicus* subsp. *melaninogenicus* ATCC 25845 (2), *B. asaccharolyticus* NCTC 9337 (3), *B. asaccharolyticus* ATCC 25260 (4), *B. melaninogenicus* subsp. *intermedius* ATCC 25261 (5), *C. ochracea* ATCC 27872 (6), or *H. influenzae*

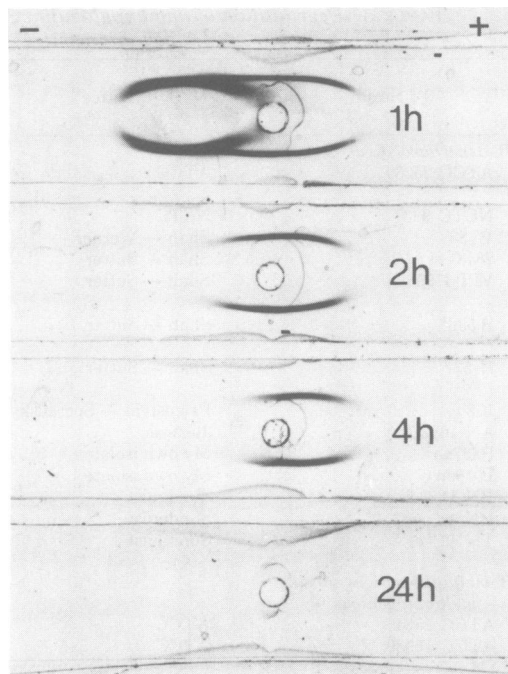


FIG. 2. Immunoelectrophoretic pattern of a polymeric IgA1 paraprotein after incubation with *B. melaninogenicus* subsp. *intermedius* A452 for 1, 2, 4, and 24 h. Antiserum: rabbit anti S-IgA. An identical pattern of gradually progressing degradation was observed for IgA2 paraprotein incubated with strain A452.

tivity was detectable (Fig. 1). Incubation of IgA2 with the remaining strains of these taxa, strains of *B. melaninogenicus* subsp. *melaninogenicus*, and the *Capnocytophaga* species did not result in any detectable cleavage of the immunoglobulin (Fig. 1).

Incubation of purified colostrum S-IgA with strains that caused degradation of both subclasses of IgA resulted in complete degradation of the substrate (see below). When incubated with strains that cleaved only IgA1, part of the S-IgA remained intact in all cases, in concordance with previous observations of other IgA1 protease-producing bacteria (14, 22).

Polyclonal IgG was cleaved by 32 of the examined strains (Table 1). These IgG-cleaving strains included all strains of the *Capnocytophaga* species and all strains of *B. asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius* capable of causing extensive degradation of IgA1. In contrast, none of the strains of *B. mel-*

62701 (7). Antisera: rabbit anti-S-IgA was used in (A), and mono-specific antisera to  $\alpha$ - (a- $\alpha$ ) and  $\kappa$ -chains (a- $\kappa$ ) were used in Fig. (B).

*aninogenicus* subsp. *melaninogenicus* induced detectable cleavage of IgG. Among the IgG-cleaving strains, all *Capnocytophaga* strains and one strain each of *B. asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius* cleaved the immunoglobulin to yield two separate precipitation lines by immunoelectrophoresis (Fig. 3). All remaining strains caused extensive degradation of IgG, the result being that no antigenic reactivity could be detected.

**Sensitivity to metal chelator.** Comparative examinations by immunoelectrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of IgA1 and IgA2 cleavage were carried out with and without ethylenediaminetetraacetic acid (EDTA) added to the substrate solution. The presence of 75 mM EDTA completely inhibited cleavage of IgA1 by strains of *Capnocytophaga*. Likewise, strains of *B. asaccharolyticus* and *B. melaninogenicus*, which normally caused complete degradation of IgA1 and IgA2, were unable to induce detectable degradation of these substrates in the presence of 75 mM EDTA. In contrast, IgA1 cleavage by strains of *B. melaninogenicus* subsp. *melaninogenicus* and *B. asaccharolyticus*, which left the Fab fragment intact (see below), were unaffected by EDTA added to the substrate.

**Properties of cleavage products.** Immunoelectrophoresis of IgA1 cleaved by strains of *B. melaninogenicus* subsp. *melaninogenicus* and *Capnocytophaga* revealed two separate fragments with different electrophoretic mobilities and antigenic determinants. One fragment, which had a slow electrophoretic mobility, reacted strongly with anti- $\alpha$ -chain serum but not with anti-L-chain ( $\kappa$ ) serum (Fig. 1). The second fragment reacted strongly with anti-L-chain serum and weakly with anti- $\alpha$ -chain serum. This

pattern closely resembled that observed with IgA1 that had been incubated with *H. influenzae* IgA1 protease. The same resemblance among IgA1 cleaved by *B. melaninogenicus* subsp. *melaninogenicus*, *Capnocytophaga* spp. and *H. influenzae* was observed by sodium dodecyl sulfate-polyacrylamide gel analysis (Fig. 4). All three samples contained two major fragments with apparent molecular weights between 58,000 and 68,000, which, upon reduction, yielded smaller fragments with molecular weights between 25,000 and 29,000. On the bases

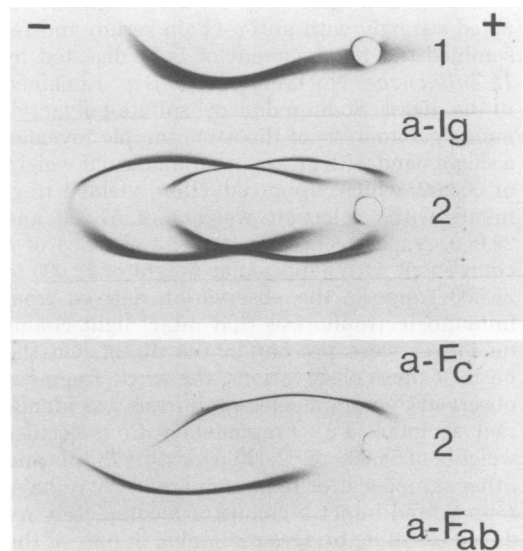


FIG. 3. Immunoelectrophoretic pattern of polyclonal IgG after incubation with buffer (1), and with cells of *C. ochracea* ATCC 27872 (2). Antisera: rabbit anti-human immunoglobulin (a-Ig), rabbit anti-Fc $_{\gamma}$  (a-Fc), and rabbit anti-Fab $_{\gamma}$  (a-Fab).

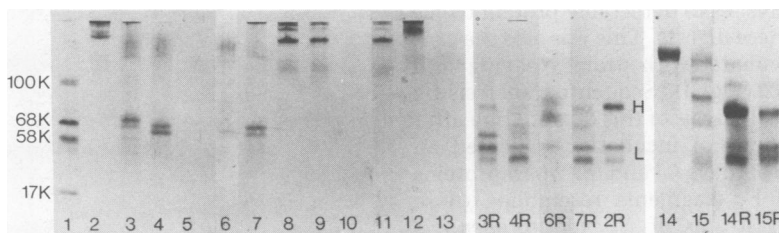


FIG. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified immunoglobulins before and after digestion by bacteria. Lane 1, Standard protein. Polymeric IgA1 paraprotein incubated with buffer (lane 2), *H. influenzae* 62701 (lane 3), *B. melaninogenicus* subsp. *melaninogenicus* ATCC 25845 (lane 4), *B. intermedius* ATCC 25261 (lane 5), *B. asaccharolyticus* ATCC 25260 (lane 6), *C. ochracea* ATCC 27872 (lane 7). IgA2 paraprotein incubated with buffer (lane 8), *B. melaninogenicus* subsp. *melaninogenicus* ATCC 25845 (lane 9), *B. intermedius* ATCC 25261 (lane 10), and *C. ochracea* ATCC 27872 (lane 11). Colostral S-IgA incubated with buffer (lane 12), and *B. intermedius* ATCC 25261 (lane 13). Polyclonal IgG incubated with buffer (lane 14), and *C. ochracea* ATCC 27872 (lane 15). Selected samples are also shown in reduced form (lanes designated "R"). H and L respectively indicate heavy and light chains of reduced IgA1 paraprotein (Car.). Molecular weights are given on the left.

of antigenic determinants, the observed resemblance with fragments of IgA1 cleaved by *H. influenzae* (14), and the determined molecular weights in unreduced and reduced form, the two fragments of IgA1 cleaved by *B. melaninogenicus* subsp. *melaninogenicus* and *Capnocytophaga* spp. were identified as Fc<sub>α</sub> and Fab<sub>α</sub>, respectively.

The single fragment observed by immunoelectrophoresis of IgA1 digested by four *B. asaccharolyticus* strains and one strain of *B. melaninogenicus* subsp. *intermedius* (Table 1) had an electrophoretic mobility distinctly different from that of native IgA1 (Fig. 1). The fragment reacted strongly with anti- $\alpha$ -chain serum and resembled the Fc<sub>α</sub> fragment of IgA1 digested by *H. influenzae*. No L-chain reactivity remained in the digest. Sodium dodecyl sulfate-polyacrylamide gel analysis of the same sample revealed a single band with an apparent molecular weight of 60,000, which, upon reduction, yielded fragments with molecular weights of 57,000 and 29,000, respectively (Fig. 4). The absence of a component with a molecular weight of 22,000 to 25,000 supports the observation derived from immunoelectrophoresis that intact light chains no longer were present in the digest. On the basis of these observations, the single fragment observed by immunoelectrophoresis was identified as intact Fc<sub>α</sub>. Fragments with molecular weights of 54,000 to 62,000 persisting in this and other samples after reduction are likely to have represented intact  $\alpha$ -chains or incompletely reduced disulfide bridges remaining in part of the Fc<sub>α</sub> fragments.

The above-mentioned suggestion of an extensive degradation of the IgA1 and IgA2 substrates induced by some strains of *B. asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius* was confirmed by sodium dodecyl sulfate-polyacrylamide gel analysis. No detectable protein bands remained in these digests. This was also the case with S-IgA incubated with strains cleaving both IgA1 and IgA2 (Fig. 4). Sequential analysis by immunoelectrophoresis of this degradation after increasing periods of incubation indicated an initial cleavage of the IgA1 and IgA2 paraproteins into Fab and Fc fragments resembling those observed with strains of *B. melaninogenicus* subsp. *melaninogenicus* (Fig. 2).

The two fragments of polyclonal IgG resulting from incubation with strains of *Capnocytophaga* and one strain of *B. asaccharolyticus* showed distinctly different electrophoretic mobilities (Fig. 3). The two fragments showed strong specific reactivity with anti-Fab, and anti-Fc<sub>γ</sub> sera, respectively. However, because several fragments could be detected by sodium dodecyl sulfate-polyacrylamide gel analysis, a definitive

characterization of the cleavage pattern requires further studies (Fig. 4).

**Degradation of IgA1 by subgingival dental plaque.** From four patients with destructive periodontitis, subgingival plaque was collected with a curette and suspended in 50  $\mu$ l of a 2-mg/ml solution of IgA1 paraprotein. Immunoelectrophoresis of the samples after incubation for 18 h at 35°C demonstrated extensive degradation of the IgA1 substrate in all four samples. However, the patterns observed in the four samples were mutually different, ranging from several distinctive cleavage fragments (Fig. 5) to complete disappearance of antigenic reactivity.

## DISCUSSION

This study demonstrated that several species of anaerobic oral bacteria, which have been implicated as principal etiological agents in destructive periodontal disease, were capable of degrading human IgA1 and IgG. Furthermore, this is the first demonstration of proteolytic degradation of human IgA2 by bacteria.

Several different patterns of immunoglobulin degradation were observed among the 45 strains examined. The selective cleavage of IgA1 induced by strains of *B. melaninogenicus* subsp. *melaninogenicus* and *Capnocytophaga* closely resembled that previously observed with strains of *N. gonorrhoeae*, *N. meningitidis* (21), *H. influenzae*, *S. pneumoniae* (14, 15), *S. sanguis* (20), and *S. mitior* (12). Immunochemical analysis of the cleavage products support this resem-

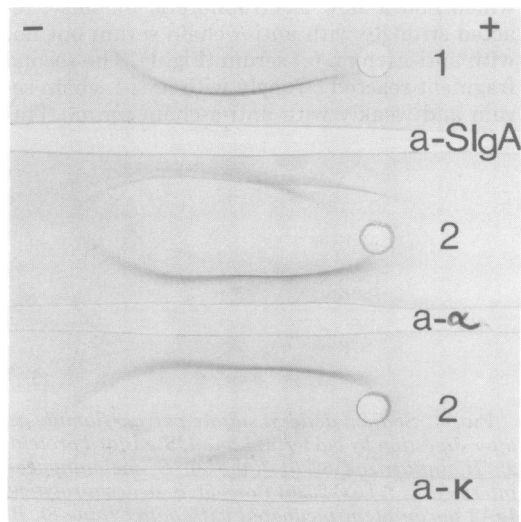


FIG. 5. Example of degradation of a polymeric IgA1 paraprotein after incubation for 18 h with subgingival plaque. (1) Buffer control; (2) degraded IgA1. Antisera: rabbit anti S-IgA (a-SIgA) and mono-specific antisera to  $\alpha$ - (a- $\alpha$ ) and  $\kappa$ - (a- $\kappa$ ) chains.

blance and indicate that strains of *B. melaninogenicus* subsp. *melaninogenicus* and the *Capnocytophaga* species cleaved the IgA1 molecule at the hinge region of the  $\alpha$ -chain to yield intact Fab and Fc fragments. This conclusion was based on the observed molecular weights of the fragments in unreduced and reduced forms, their reaction with mono-specific antisera against L- and H-chains, and their immunochemical similarity to fragments derived from IgA1 cleaved by *H. influenzae* IgA1 protease, the exact cleavage site of which has been established (13). Furthermore, the fact that the proteases produced by these bacteria were unable to cleave IgA2 suggests that the susceptible site(s) in the hinge region of the  $\alpha$ 1-chain is within the 13-amino acid sequence, which is deleted in the  $\alpha$ 2-chain (22). The finding that the IgA1 cleavage induced by *Capnocytophaga* spp. was EDTA sensitive, in contrast to that induced by *B. melaninogenicus* subsp. *melaninogenicus*, confirm our previous observation (13) that both metal chelator-sensitive and -resistant bacterial IgA1 proteases exist.

In contrast to previously described IgA1 protease-producing bacteria, the *Capnocytophaga* strains also cleaved polyclonal IgG. Preliminary immunochemical examination of the cleavage products indicate that cleavage took place in the hinge region of the  $\gamma$ -chain.

The strains of *B. asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius* differed from the above-mentioned taxa and from all previously described IgA1 protease-producing bacteria by inducing more extensive degradation of the IgA1 substrate. However, among these strains, differences were observed in the pattern of IgA1 degradation and in the ability to decompose IgA2 and IgG.

The majority of strains of *B. asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius* digested both human IgA1 paraprotein and polyclonal serum IgG into small-molecular-weight fragments that were nondetectable by immunochemical means. Ten of these strains, in addition, caused complete degradation of IgA2 paraprotein (Table 1). The potent proteolytic activity of these 10 strains was also demonstrated by their ability to cause complete degradation of colostral S-IgA. The pattern of sequential degradation of both IgA1 and IgA2 observed with these strains suggests an initial cleavage of the IgA molecules in the hinge region with subsequent extensive degradation of the Fab and Fc fragments. The complete absence of cleavage in the presence of EDTA indicates that the enzyme(s) responsible for the initial opening of the molecule or, alternatively, all involved enzymes, was metal chelator-sensitive proteases. Purifi-

cation and characterization of the proteolytic enzymes produced by the responsible strains of *B. asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius* are required to elucidate the individual processes leading to the complete degradation of IgA1, IgA2, and polyclonal IgG. Such studies may identify a specific IgA2 protease.

The production of several proteolytic enzymes was also evident in the strains of *B. asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius*, which caused complete degradation of the Fab part of the IgA1 molecule but left the Fc part apparently intact. The molecular weight of this remaining Fc<sub>2</sub> fragment and the selective cleavage of IgA1 indicate that these organisms possessed a specific IgA1 endopeptidase of the traditional type. The inability of EDTA to inhibit the proteolytic attack indicates that the enzyme was a metal chelator-resistant protease such as those observed in *B. melaninogenicus* subsp. *melaninogenicus* and *H. influenzae*. However, in contrast to the latter two organisms, these strains of *B. asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius* produced additional proteolytic enzymes responsible for the complete degradation of polyclonal IgG and the Fab part of the IgA1 molecule. The fact that these strains left the Fc part of the IgA1 molecule apparently intact suggests that they lacked exopeptidases capable of attacking the residue at the amino side (Fc) of the initial splitting point in the hinge region.

The significantly different patterns of cleavage of immunoglobulins among the strains of *B. asaccharolyticus* and *B. melaninogenicus* subsp. *intermedius* probably reflect the heterogeneity previously observed, in particular, within *B. asaccharolyticus* (4, 25, 37). Recently, this heterogeneity resulted in a proposal to assign "oral-type" strains of *B. asaccharolyticus*, which are characterized by a relatively low guanine-plus-cytosine content of deoxyribonucleic acid, to a separate species, *B. gingivalis* (4). The strains included in the present study encompassed both oral-type and non-oral-type strains (25, 37). However, no correlation between pattern of immunoglobulin degradation and subtype of the strains could be established. Complete degradation of IgA1, IgA2, and IgG was observed with both oral and non-oral isolates.

It is remarkable that among more than 75 bacterial species examined, the ability to cleave IgA1 is restricted to a few groups of bacteria that are etiological agents in three important infectious diseases: meningitis (*N. meningitidis*, *H. influenzae*, and *S. pneumoniae*), gonorrhoea (*N. gonorrhoeae*) (14, 15, 21), and destructive periodontal disease (*B. asaccharolyticus*, *B. melaninogenicus*, and *Capnocytophaga* spp.).

In addition, some strains of the two streptococcal species, *S. sanguis* and *S. mitior*, which initiate dental plaque formation, produce IgA1 protease (12, 20). The disease association of these otherwise unrelated IgA1 protease-producing bacteria strongly suggests a pathogenic function of the IgA1-cleaving enzymes. With respect to bacterial meningitis, this hypothesis is supported by the findings that nonpathogenic species of *Neisseria* and *Haemophilus* lack IgA1 protease activity (14, 17) and that two bacterial species responsible for meningitis and other invasive diseases in swine and cattle cleave porcine and bovine S-IgA, respectively (14; unpublished data).

Although the final proof is still lacking, there is considerable indirect evidence showing that *B. asaccharolyticus*, *B. melaninogenicus*, and *Capnocytophaga* spp. are principal etiological agents in the destructive forms of periodontal disease (18, 19, 27, 28, 30, 31, 33). The former two species, furthermore, seem to play a key role in mixed anaerobic infections, including purulent periapical dental abscesses (7, 32). A large proportion of the strains included in this study originated from periodontal disease and were capable of degrading IgA1, polyclonal IgG, and, in several cases, IgA2. The presence of IgA1-degrading enzymes in subgingival plaque was also clearly demonstrated when IgA1 was incubated with samples of whole plaque collected from gingival pockets of patients with periodontitis.

Bacterial meningitis, gonorrhoea, and destructive periodontal disease are characterized by the apparently uninhibited penetration of bacteria or bacterial products through the mucosal barrier. In periodontal disease, it is the penetration of bacterial proteolytic enzymes, toxic substances, and strong immunopotentiating bacterial components through the junctional epithelium of the gingival sulcus that is held responsible for the breakdown of the periodontium and the ensuing loss of teeth (29, 34, 35). Considerable literature exists about the penetration of molecules through the junctional epithelium of the gingival sulcus (for reviews, see references 24, 34, 35). Although definitive conclusions are difficult, the combined data from such studies suggest that the junctional epithelium allows the penetration of molecules of up to at least 70,000 molecular weight. However, recent studies of the effect of preexisting antibody on the penetration of antigens through a variety of oral and nonoral epithelial surfaces show that both local and serum-derived antibodies of the IgA and IgG classes can limit the entry and spread of homologous antigen (1, 3, 5, 8, 16, 23, 36, 38, 39). In the periodontal area, this function is maintained by

immunoglobulins present both in the gingival crevice fluid and in the gingival tissues.

Although there is conflicting information on the exact relative amounts of IgA and IgG in gingival fluid (2, 3, 10, 26, 34), there is agreement that these immunoglobulin classes predominate. Their relative concentrations and the absence of secretory component from the IgA (3, 10) indicate that the gingival fluid should be considered an exudate. This would suggest that the vast majority of the IgA is of the IgA1 subclass. In this respect, it seems pertinent to emphasize that the extensive degradation of both IgA and IgG, which may be induced by bacteria inhabiting the gingival crevice, is bound to have a profound influence on quantitative determinations of immunoglobulins in dental plaque and gingival crevice fluid.

In the gingival tissues, IgG is clearly predominating (3, 34). However, Brandtzaeg and Tolo (3) have argued that IgA particularly is conducive to health in this area because of the inability of immune complexes that involve IgA to initiate complement reactions and to cause a release of lytic enzymes from inflammatory cells.

On the basis of the results presented in this paper, it seems conceivable that colonization of the subgingival area by bacteria such as *B. asaccharolyticus*, *B. melaninogenicus*, and *Capnocytophaga* spp., which have the potential to induce extensive degradation of all immunoglobulins present in the area, can cause a local paralysis of the immune defence mechanisms, thereby facilitating the penetration and spread of potentially toxic substances, lytic enzymes, and antigens released by the entire subgingival microflora.

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